

# Analytical Biochemistry

*An International Journal*

EDITOR: *Alvin Nason*

## ADVISORY BOARD:

|                            |                            |                               |
|----------------------------|----------------------------|-------------------------------|
| <i>Geoffrey Ackers</i>     | <i>William Z. Hassid</i>   | <i>Kazuo Okunuki</i>          |
| <i>Norman G. Anderson</i>  | <i>Osamu Hayaishi</i>      | <i>Alexander Rich</i>         |
| <i>Edo Antonini</i>        | <i>Edward C. Heath</i>     | <i>David Rodbard</i>          |
| <i>Geert Ashwell</i>       | <i>Leslie Hellerman</i>    | <i>Gerhard Schmidt</i>        |
| <i>Lemuel P. Bessman</i>   | <i>C. H. W. Hirs</i>       | <i>Michael Sela</i>           |
| <i>John Black</i>          | <i>Evan C. Horning</i>     | <i>Esmond E. Snell</i>        |
| <i>Geert M. Bock</i>       | <i>Rollin D. Hotchkiss</i> | <i>Herbert A. Sober</i>       |
| <i>Geert L. Bowman</i>     | <i>Walter L. Hughes</i>    | <i>Sol Spiegelman</i>         |
| <i>Ludwig Brand</i>        | <i>William B. Jakoby</i>   | <i>Paul K. Stumpf</i>         |
| <i>E. Braunstein</i>       | <i>Nathan O. Kaplan</i>    | <i>Charles A. Thomas, Jr.</i> |
| <i>Linus Pauling</i>       | <i>Ephraim Katchalski</i>  | <i>Sidney Udenfriend</i>      |
| <i>Alfred Chrambach</i>    | <i>Irving Klotz</i>        | <i>Bert L. Vallee</i>         |
| <i>Henry Colowick</i>      | <i>Luis F. Leloir</i>      | <i>Samuel B. Weiss</i>        |
| <i>Jack Eisenberg, Jr.</i> | <i>Feodor Lynen</i>        | <i>Theodor Wieland</i>        |
| <i>Frances R. Fresco</i>   | <i>Brian J. McCarthy</i>   | <i>George Wolf</i>            |
| <i>Isidor Ginsburg</i>     | <i>Vincent Massey</i>      | <i>W. A. Wood</i>             |
| <i>John Gonsky</i>         | <i>Alan H. Mehler</i>      | <i>Lemuel D. Wright</i>       |
| <i>John O. Halvorson</i>   | <i>Bruce Merrifield</i>    | <i>David A. Yphantis</i>      |
|                            | <i>Marshall Nirenberg</i>  |                               |

VOLUME 58

1974

ACADEMIC PRESS  
New York and London



*A Subsidiary of Harcourt Brace Jovanovich, Publishers*

Copyright © 1974 by Academic Press, Inc.

All Rights Reserved

No part of this publication may be reproduced or transmitted in any form, or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the copyright owner.

# CONTENTS OF VOLUME 58

NUMBER 1, MARCH 1974

|                                                                                                                                                                                                      |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| A. J. SCISM, J. N. BEMILLER, AND A. L. CASKEY. Determination of 2,4-Dihydroxy-1,4(2H)-benzoxazin-3-one Glucosides in Corn ( <i>Zea mays</i> L.) . . . . .                                            | 1   |
| L. A. KIESOW, J. B. SHELTON, AND J. W. BLESS. The Determination of O <sub>2</sub> -Dissociation Curves in Hemoglobin Solutions with a Liquid Fluorocarbon O <sub>2</sub> -Transport System . . . . . | 14  |
| MICHAEL MAYER, SARA KHAISIS, AND ELEAZAR SHAFRIR. Determination of Trypsin by Its Accelerating Effect on the Onset of Trypsinogen Activation . . . . .                                               | 25  |
| SHASHIAHU SHECHTER. Separation of Proteins by High-Speed Pressure Liquid Chromatography . . . . .                                                                                                    | 30  |
| SCHUYLER D. SEELEY AND LOYD E. POWELL. Gas Chromatography and Detection of Microquantities of Gibberellins and Indoleacetic Acid as Their Fluorinated Derivatives . . . . .                          | 39  |
| DAVID B. BYLUND AND GEORGE BRUENING. Prediction of Centrifugation Times for Equilibrium and Velocity Sedimentation on Various Gradients . . . . .                                                    | 47  |
| E. M. GREGORY AND I. FRIDOVICH. Visualization of Catalase on Acrylamide Gels . . . . .                                                                                                               | 57  |
| M. E. ELDEFRAWI AND H. C. FERTUCK. A Rapid Method for the Preparation of [ <sup>125</sup> I]α-Bungarotoxin . . . . .                                                                                 | 63  |
| E. JÜRGEN ZÖLLNER, BERND HEICKE, AND RUDOLF K. ZAHN. Human Serum Deoxyribonuclease Assay in [ <sup>3</sup> H]DNA-Polyacrylamide Gels without Staining Artifacts . . . . .                            | 71  |
| KAZUO YOSHIDA, NOBUKO IINO, IKUE KOGA, AND KEITARO KATO. Demonstration of β-Glucuronidase in Disk Electrophoresis by Means of 5-Bromoindol-3-yl-β-D-glucuronide . . . . .                            | 77  |
| WILLIAM MEINKE, DAVID A. GOLDSTEIN, AND MARK R. HALL. Rapid Isolation of Mouse DNA from Cells in Tissue Culture . . . . .                                                                            | 82  |
| M. R. V. MURTHY AND HUGUETTE ROUX. Recovery of Amino Acids and tRNA after Scintillation Counting of Radioactive Aminoacyl-tRNA in Filter Paper Strips . . . . .                                      | 89  |
| JÖRG URBAN, PETER ZIMMER, AND GERHARD SCHREIBER. Immunoprecipitation is Inappropriate for the Isolation of Radiochemically Pure Albumin from Tissues . . . . .                                       | 102 |
| NAM HEA LEE AND IRVING M. SHAPIRO. A Method for Isolating Respiring Mitochondria from Epiphyseal Cartilage . . . . .                                                                                 | 117 |
| R. CHABY, S. R. SARFATI, AND L. SZABÓ. Colorimetric Estimation of 3-Deoxy-D-manno-octulosonic Acid in Oligosaccharides with Diphenylamine . . . . .                                                  | 123 |



|                                                                                                                                                                                         |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| VINCENZO ZAPPIA, PATRIZIA GALLETTI, MARIA CARTENÍ-FARINA, AND<br>LUIGI SERVILLO. A Coupled Spectrophotometric Enzyme Assay<br>for Methyltransferases . . . . .                          | 130 |
| W. L. VAN ES AND W. S. BONT. The Simultaneous Measurement<br>of Densities and Absorbances in Sucrose Gradients, Applied to<br>Zonal Centrifugation . . . . .                            | 139 |
| L. P. T. M. ZEVENHUIZEN. Spectrophotometric Assay of Long-<br>Chain Unsaturated and Hydroxy Fatty Acids in Concentrated<br>Sulfuric Acid . . . . .                                      | 146 |
| E. K. MATTHEWS, O. H. PETERSEN, AND J. A. WILLIAMS. Analysis<br>of Tissue Amylase Output by an Automated Method . . . . .                                                               | 155 |
| DAVID J. COX AND ALLEN T. ANSEVIN. Rapid Alignment of the<br>Interference Mask in the Ultracentrifuge . . . . .                                                                         | 161 |
| ERIK SLINDE, HÅVARD STORETVEDT, FINN STRAND, AND TORGEIR FLAT-<br>MARK. A Low-Cost Integrator for Preparative Ultracentrifuges . . . . .                                                | 170 |
| DANIEL V. SANTI AND ROBERT T. ANDERSON, JR. A DEAE-Cellulose<br>Filter Disk Assay for Aminoacyl-tRNA . . . . .                                                                          | 175 |
| P. W. TRUDGILL. A Simple Oxygen Electrode for Use in a Spectro-<br>photometer . . . . .                                                                                                 | 183 |
| M. P. JACOB SPURGEON ANANDARAJ AND JOSEPH D. CHERAYIL. A<br>Simple Method for Large-Scale Preparation of Chick Embryo<br>tRNA . . . . .                                                 | 190 |
| PETER G. N. JEPPESEN. A Method for Separating DNA Fragments<br>by Electrophoresis in Polyacrylamide Concentration Gradient<br>Slab Gels . . . . .                                       | 195 |
| KARL E. WEIBEL, JUAN-R. MOR, AND ARMIN FIECHTER. Rapid<br>Sampling of Yeast Cells and Automated Assays of Adenylate,<br>Citrate, Pyruvate and Glucose-6-phosphate Pools . . . . .       | 208 |
| E. WEIDEKAMM, D. F. H. WALLACH, P. NEURATH, R. FLÜCKIGER, AND<br>J. HENDRICKS. Analysis of Polyacrylamide Gel Electrophoresis<br>Through the PIQUANT Image Recognition System . . . . . | 217 |
| UELI SCHIBLER AND RUDOLF WEBER. A New Method for the Isola-<br>tion of Undegraded Nuclear and Cytoplasmic RNA from Liver<br>of <i>Xenopus</i> Larvae . . . . .                          | 225 |
| CHARLES D. YEGIAN. A Procedure for the Measurement of Intra-<br>cellular Deoxyribonucleoside Triphosphate Pools by Thin Layer<br>Chromatography . . . . .                               | 231 |
| KRISTIAN S. BJERVE, LUDVIG N. W. DAAE, AND JON BREMER. The<br>Selective Loss of Lysophospholipids in Some Commonly Used<br>Lipid-Extraction Procedures . . . . .                        | 238 |
| MICHAEL J. BLACK AND RICHARD B. BRANDT. Spectrofluorometric<br>Analysis of Hydrogen Peroxide . . . . .                                                                                  | 246 |
| JOHN I. PETERSON, WALTER S. FRIAUF, AND STEPHEN B. LEIGHTON.<br>A High Precision Fluorometer for Biochemical Measurements . . . . .                                                     | 255 |

|                                                                                                                                                                                       |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| ROY H. HAMMERSTEDT. A Rapid Method for the Determination of Glycerophosphorylcholine in Rabbit and Bull Seminal Plasma                                                                | 272 |
| GEORGE FLOURET AND OSCAR HECHTER. Adsorption Chromatography of Cyclic Nucleotides on Silica Gel and Alumina Thin-Layer Sheets . . . . .                                               | 276 |
| Y. P. L. YOUNG AND R. J. YOUNG. A Method for the Recovery of Nucleic Acids from Polyacrylamide Gels . . . . .                                                                         | 286 |
| JOHN D. FINDLATER AND BRUNO A. ORSI. Assay for the Enzymic Hydrolysis of Penicillin and Acylhydroxamates . . . . .                                                                    | 294 |
| SHLOMO GROSSMAN, G. OESTREICHER, P. K. HOGUE, J. G. COBLEY, AND THOMAS P. SINGER. Microanalytical Determination of the Activities of Phospholipases A, C, and D and of their Mixtures | 301 |
| E. A. SIESS AND O. H. WIELAND. Enzymatic Synthesis of <sup>32</sup> P-Labeled Coenzyme A . . . . .                                                                                    | 310 |
| MARVA J. VOLK, RICHARD N. TRELEASE, AND HENRY C. REEVES. Determination of Malate Synthase Activity in Polyacrylamide Gels . . . . .                                                   | 315 |
| M. M. HERBST, B. G. BALTIMORE, G. BOZLER, AND H. A. BARKER. Identification and Estimation of Some Uncommon Diamino Acids . . . . .                                                    | 322 |

# SHORT COMMUNICATIONS

|                                                                                                                                               |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----|
| B. M. WATTS AND N. A. M. ESKIN. Percentage Recovery of Free Fatty Acids from Bovine Muscle Lipids by Non-chromatographic Techniques . . . . . | 331 |
| KENT PAINTER AND M. J. GEZING. The Advantage of Black Caps for Liquid Scintillation Vials . . . . .                                           | 334 |

## NUMBER 2, APRIL 1974

|                                                                                                                                                                                                                              |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| JOHN B. SWANEY, GEORGE F. VANDE WOUDE, AND HOWARD L. BACH-RACH. Sodium Dodecylsulfate-Dependent Anomalies in Gel Electrophoresis: Alterations in the Banding Patterns of Foot-and-Mouth Disease Virus Polypeptides . . . . . | 337 |
| K. WETZ, H. FASOLD, AND CH. MEYER. Synthesis of "Long" Hydrophilic, Protein-Cross-Linking Reagents . . . . .                                                                                                                 | 347 |
| WILLIAM A. AYRES, ENOCH W. SMALL, AND IRVIN ISENBERG. A Computerized Fluorescence Anisotropy Spectrometer . . . . .                                                                                                          | 361 |
| MADHU S. P. MANANDHAR AND KNOX VAN DYKE. Adenosine Tetraphosphate Analysis: Polyethyleneimine (PEI) Thin Layer Purification and Firefly-Extract Liquid Scintillation Quantitation . . . . .                                  | 368 |
| Y. S. PRASADA RAO AND JOSEPH D. CHERAYIL. Separation of Sulfur-                                                                                                                                                              |     |



|                                                                                                                                                                                                                        |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Containing Components of Transfer Ribonucleic Acid on Bio-Gel P-2 and Sephadex G-10 Columns . . . . .                                                                                                                  | 376 |
| RONALD T. BORCHARDT. A Rapid Spectrophotometric Assay for Catechol- <i>O</i> -Methyltransferase . . . . .                                                                                                              | 382 |
| DONALD L. ESHENBAUGH, DONALD SENS, AND ERIC JAMES. Rapid Sedimentation of <i>Escherichia coli</i> in the Presence of Polyethylene Glycol at 1g . . . . .                                                               | 390 |
| LAURA C. MINASIAN AND ANNE G. JABARA. A Technique for Isolating Pure, Viable Parenchymal Cells and Their Nuclei from Rat Mammary Gland . . . . .                                                                       | 395 |
| M. KAWAI AND B. L. HILLCOAT. Nonenzymatic Exchange of Tritium from [5- <sup>3</sup> H]Deoxyuridyate in the Thymidyate Synthetase Assay . . . . .                                                                       | 404 |
| KARLA L. ROEHRIG AND JOHN B. ALLRED. Direct Enzymatic Procedure for the Determination of Liver Glycogen . . . . .                                                                                                      | 414 |
| WILLIAM M. LAMKIN, JANE WADE WEATHERFORD, NANCY S. JONES, THERESA PAN, AND DARRELL N. WARD. Analysis of Methylthiohydantoins of Amino Acids by Gas-Liquid Chromatography of Their Trimethylsilyl Derivatives . . . . . | 422 |
| TAKEHISA KANEKO AND HIROSHI TERAYAMA. Quantitative Analysis of DNA in Sea Urchin Eggs and Subcellular Distribution of DNA in the Eggs . . . . .                                                                        | 439 |
| GEORGE P. STUDZINSKI AND GARY J. FISCHMAN. Activation of DNA Primer for DNA Polymerase as an Ultrasensitive Assay for a Subgroup of Enzymes with Deoxyribonuclease Activity . . . . .                                  | 449 |
| CHARLES O. BROSTROM AND CHRISTINE KON. An Improved Protein Binding Assay for Cyclic AMP . . . . .                                                                                                                      | 459 |
| H. R. GUTMANN. Isolation and Identification of the Carcinogen <i>N</i> -Hydroxy-2-fluorenylacetamide and Related Compounds by Liquid Chromatography . . . . .                                                          | 469 |
| K. T. IZUTSU, S. P. FELTON, I. A. SIEGEL, J. I. NICHOLLS, J. CRAWFORD, J. MCGOUGH, AND W. T. YODA. An Investigation of the Ionic Calcium Assay Based on the Jellyfish Protein Aequorin . . . . .                       | 479 |
| L. R. TRAVASSOS AND CELUTA O. SALES. Microbiological Assay of Carnitine . . . . .                                                                                                                                      | 485 |
| PETER BODLAENDER, DAVID D. ULMER, AND BERT L. VALLEE. Automated Determination of $\delta$ -Aminolevulinic Acid Dehydratase Activity in Human Erythrocytes . . . . .                                                    | 500 |
| DONALD H. NAMM AND JOAN P. LEADER. A Sensitive Analytical Method for the Detection and Quantitation of Adenosine in Biological Samples . . . . .                                                                       | 511 |
| GERALD L. ROWLEY AND GEORGE L. KENYON. PEI-Cellulose Thin-Layer Chromatography. Product Studies of the Creatine Kinase and Pyruvate Kinase Reactions . . . . .                                                         | 525 |

|                                                                                                                                                                                                                                  |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| D. L. KACIAN AND S. SPIEGELMAN. Use of Micrococcal Nuclease to Monitor Hybridization Reactions with DNA . . . . .                                                                                                                | 534 |
| YORAM SALOMON, CONSTANTINE LONDOS, AND MARTIN RODBELL. A Highly Sensitive Adenylate Cyclase Assay . . . . .                                                                                                                      | 541 |
| WILLIAM M. LAMKIN, NANCY S. JONES, THERESA PAN, AND DARRELL N. WARD. Application of the Flame Photometric Sulfur Detector to the Gas-Chromatographic Analysis of Trimethylsilylated Methylthiohydantoin of Amino Acids . . . . . | 549 |
| PETER BÖHLEN, STANLEY STEIN, KAZUHIRO IMAI, AND SIDNEY UDEN-FRIEND. A Simplified Protein Assay with Fluorescamine in Samples Containing Interfering Material . . . . .                                                           | 559 |
| N. NAKAI, C. Y. LAI, AND B. L. HORECKER. Use of Fluorescamine in the Chromatographic Analysis of Peptides from Proteins . . . . .                                                                                                | 563 |
| MAKOTO NAOI, Y. C. LEE, AND S. ROSEMAN. Rapid and Sensitive Determination of Sphingosine Bases and Sphingolipids with Fluorescamine . . . . .                                                                                    | 571 |
| GANES C. SEN AND HARA P. GHOSH. A Fast and Sensitive Method for the Analysis of Modified Nucleosides in tRNA . . . . .                                                                                                           | 578 |
| JAMES H. ALLISON, HARISH C. AGRAWAL, AND BLAKE W. MOORE. Effect of <i>N,N,N',N'</i> -Tetramethylethylenediamine on the Migration of Proteins in SDS Polyacrylamide Gels . . . . .                                                | 592 |
| ARIE ROSNER, MARVIN EDELMAN, AND JONATHAN GRESSSEL. Thermal Denaturation of Nucleic Acids in Polyacrylamide Gels . . . . .                                                                                                       | 602 |
| J. R. QUINN, J. G. A. BOISVERT, AND I. WOOD. Semi-Automated Ninhydrin Assay of Kjeldahl Nitrogen . . . . .                                                                                                                       | 609 |
| ROBERT H. DAVIS, JOHN H. COPENHAVER, AND MICHAEL J. CARVER. Quantitation of Stained Proteins in Polyacrylamide Gels . . . . .                                                                                                    | 615 |
| J. E. BENEYTO AND J. COLOMÉ. Direct Spectrophotometric Determination of Mucin Disulfide Linkages . . . . .                                                                                                                       | 624 |

# SHORT COMMUNICATIONS

|                                                                                                                                                                             |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| J. A. DAVISON AND G. H. FYNN. The Assay of ATP by the Luciferin-Luciferase Method. Interference by a Bacterial Phosphatase Enzyme Stable to Perchlorate Treatment . . . . . | 632 |
| J. X. KHYM. Group Separation of Ribonucleotides, Ribonucleosides, and Purine and Pyrimidine Bases on Polyacrylamide Gel Columns . . . . .                                   | 638 |
| BRUCE MORTON. Sedimentation Coefficients of Higher Polyosomes From Rat Liver and the Localization of Polysomes Not Resolved by Gradient Analysis . . . . .                  | 642 |
| C. DENNISON. Differential Rates of Passage of Amino Acids Through an Ultrafiltration Membrane . . . . .                                                                     | 646 |

|                                                                                                                                               |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----|
| KARL BLAU AND CYRIL S. DODGE. Continuous Nephelometric<br>Monitoring of Glycosaminoglycans Eluted From Chroma-<br>tographic Columns . . . . . | 650 |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----|

|                   |     |
|-------------------|-----|
| ERRATUM . . . . . | 655 |
|-------------------|-----|

|                        |     |
|------------------------|-----|
| AUTHOR INDEX . . . . . | 656 |
|------------------------|-----|

Subject Index for Volume 58 will appear in the December issue as  
part of a cumulative index for the year 1974.